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THE EFFECT OF LOW DENSITY ON THE DEVELOPMENT OF SIMULTANEOUS HERMAPHRODITISM IN MALE *CAPITELLA* SPECIES I (POLYCHAETA)

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Abstract

In this study it was shown that male *Capitella* species I may develop into simultaneous hermaphrodites if reared in isolation or at low densities in the laboratory. Since hermaphroditic development is rare in high density non-inbred cultures but becomes frequent in inbred lines which have been selected for high male sex ratios, the ability to become hermaphroditic seems to have a genetic basis. It is also suggested that low density may trigger the hermaphroditic development of males through the absence of animals with female gonads. Excess food resources which could be assimilated at low density and may be directed into the development of a second set of reproductive structures and female gametes, could also be a factor in the male-to-hermaphrodite switch. *Capitella* sp. I has an opportunistic lifestyle which could accommodate such a sexual adaption. The larvae are widely dispersed which would often cause adults to be at low density where the advantage of being hermaphroditic is well known.

Introduction

Much of the scientific interest in the genus *Capitella* stems from its "opportunistic" life history (Grassle and Grassle, 1974) which has made it an important indicator of environmental disturbance in the benthos (Henrikkson, 1969; Pearson and Rosenberg, 1978; Sanders *et al.,* 1980). Formerly known as *Capitella capitata,* electrophoresis has shown it to be a complex of at least ten sibling species (Grassle and Grassle, 1976, 1977; Grassle, 1980). The different species are morphologically similar but differ in life histories and reproductive strategies. The most opportunistic species, which is distinguished by larvae which remain in the planktonic stage for several hours, is *Capitella* species I. This species is the main one under consideration here.

Males, females, and hermaphrodites are found in natural populations or *Capitella* sp. I. Hermaphrodites are found only infrequently, appearing when mature males develop female gonads. Families raised in the laboratory from individual eggcases have extremely variable sex ratios and successful selection can be made for inbred lines with a high proportion of males and females (Grassle, 1980). Both facts suggest that there may be a polygenic sex determining mechanism (Kossig, 1964). If selection is made for a highly male sex ratio, the proportion of hermaphrodites that develop will also increase. The discovery that motivated this research was that young non-inbred males of species I and II developed female gonads, thus becoming hermaphrodites, if reared in isolation. Previously, hermaphrodites were observed to develop in old, high density, non-inbred laboratory cultures only. Hermaphrodites are never

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found in *Capitella* species IIIa and all individuals of species III are hermaphroditic (Grassle, 1980).

This developmental response to isolation leads to several questions concerning the proximate stimuli causing hermaphroditism and its ultimate role in the overall reproductive strategy of the animal. The first question examined here was whether these young hermaphrodites function simultaneously as males and females. Secondly, the effects of density on hermaphroditic development were investigated. This is particularly interesting when the adaptive significance of the mixture of reproductive modes is considered. Low density is frequently cited as an important factor in the evolution of hermaphroditism (Tomlinson, 1966; Ghiselin, 1969; Maynard Smith, 1978; Borgia and Blick, 1981). Since *Capitella* may have a polygenic sex determining mechanism, it is also of interest to see how potential differences in degree of maleness and femaleness, caused by differences in sex determining alleles, may affect the rate of development of hermaphroditism in isolated males.

Materials and Methods

Two stations in New Bedford Harbor, Massachusetts have been sampled at regular intervals with 1/25 m^2 Van Veen grab from November 1981 to the present. The first, Sewer West in the outer harbor, has had low densities of *Capitella* sp. I (<0.1 worms/cm^2) and occasionally a few individuals of *Capitella* sp. III, on each sampling occasion. Station 3 in the inner harbor has revealed densities of *Capitella* spp. I and II of 4 to 6 worms/cm^2. Two samples from Sewer West and one sample from Station 3 containing hermaphrodites were sexed and subsequently sacrificed and identified to species by electrophoresis.

Males of several sibling species of *Capitella* were isolated from a field collection from the Woods Hole Sewer Outfall (6-1-81), and from a laboratory aquarium (13-1-81) with a population of *Capitella* derived from larvae entering the aquarium in the unfiltered sea water lines. Individual males were kept in filtered sea water at 15°C, fed to excess, and examined for macroscopic evidence of oögenesis at approximately weekly intervals up to 34 days. Survivors were identified to species by electrophoresis.

All of the animals used in the following experiments were *Capitella* species I and originated from laboratory stocks at the Marine Biological Laboratory, Woods Hole, Massachusetts in 1980. They were cultured at 20°C in plastic or glass containers of various sizes in a medium of mud and sea water. The mud, which supplies the food source for these animals, was collected from several intertidal sites on the eastern shore of Nova Scotia. To prepare the mud it is sieved through screens of standard size 20 (841 μ) and then frozen until use. The mud and sea water were replaced at weekly intervals in all experiments. No attempt was made to standardize the nutrients available to each animal. Food was always added in excess.

(1) To check whether hermaphrodites are functional as males and females simultaneously, 16 40-day-old males from a non-inbred line were isolated. All had become fully developed hermaphrodites after 25 days of isolation; at that time 7 were paired with 7 known males and 7 more were paired with 7 known females. The females had not been in contact with any males for 2 weeks prior to the experiment so there was a low possibility of sperm storage. Pairs were checked for eggcases at weekly intervals.

(2) Sibling males from many different families were used to determine the effect of density on hermaphroditic development. Here the term family will be used to mean a group of animals that are siblings. Families of known age were reared separately

at different densities from 0.02 worms/cm^2 to 0.19 worms/cm^2. Worms/cm^2 is used as a measure of density since *Capitella* sp. I generally feeds near the surface. Each of the family groups were sexed at weekly intervals for a period of 6 weeks. Hermaphrodites were not removed from the cultures as they developed. The influence of age of the families and their densities on the resulting proportions of hermaphrodites were analyzed by multiple regression.

(3) Variations in the time taken by isolated males to become hermaphrodites were studied using 112 males from 6 non-related families. All animals were 66–78 days-old. Fifty-six males came from 3 families which had more females than males. At the time of sexing the ratios of these 3 families were 4 ♂:7 ♀:1 juvenile, 60 ♂:69 ♀:1 juvenile and 32 ♂:41 ♀:19 juveniles. The original number of larvae set out in these families were 30, 200, and 100 respectively. The rest of the males came from families with a very high proportion of males and were, therefore, perhaps different from the others in their sex determining genes. The sex ratios of this second group of families were 65 ♂:2 ♀:8 juveniles, 20 ♂:2 ♀:9 juveniles and 76 ♂:8 ♀:8 juveniles. The original number of larvae set out in these families were 100, 50, and 100 respectively. All the males were isolated under identical conditions and checked for the development of female gonads every 2 days for 38 days. At that time the number of hermaphrodites was constant. Later, several of the males which switched to hermaphrodites early, after less than 9 days of isolation, were cultured together and a mixed batch of the resulting larvae were allowed to develop. At 69 days of age, 32 males from this group of offspring were isolated in the same manner as their parents and observed for 20 days.

Results

Hermaphrodites are found at very low densities in the field. In all the *Capitella* individuals examined at capture from Sewer West and Station 3 field stations, and that were subsequently identified electrophoretically as *Capitella* sp. I or II, only four hermaphrodites belonging to *Capitella* sp. I, were found (Table I). Table II shows that both *Capitella* sp. I and II may develop into hermaphrodites when isolated.

In the experiment to check for the functionality of simultaneous hermaphroditism, 5 of the 7 females paired with hermaphrodites were found in fertile eggcases within 14 days after the cultures were set up. There had also been successful fertilization of all hermaphrodites that had been cultured with males, demonstrating that hermaphrodites that are induced to develop through isolation can function as simultaneous hermaphrodites.

Figure 1 shows the relationship between density and the proportion of males turning to hermaphrodites in the all-male mass cultures of varying density. The multiple regression performed on the results shows that both density and age have

Table I

Number of Capitella *sp. I hermaphrodites found in 3 field samples of varying density*

Station/Sampling date	Density Worms/cm^2 *Capitella* spp.	Number of *Capitella* sp. 1 hermaphrodites	Total *Capitella* spp. individuals
Sewer West/21-12-81	0.015	2	6
Sewer West/28-6-82	0.045	1	18
Station 3/2-12-82	4.079	1	302

TABLE II

Number of Capitella *spp. I, II, and IIIa males switching to hermaphrodites and the time in which they switched under isolated conditions*

Source population	Number of *Capitella* males isolated	Number of *Capitella* dying in 34 days without switching	Number of *Capitella* of spp. I, II, and IIIa	Number switching to ♀ in 34 days or less	Mean days to switch	Range
Woods Hole	26	6	2 sp. I	2	—	14–27
Sewer			17 sp. II	15	25.7	13–34
Outfall			1 sp. IIIa	0	—	—
Aquarium	8	1	7 sp. I	7	22.0	13–27

a significant effect on the proportion of males turned to hermaphrodites (ANOVA F-ratio = 15.06, $P < .05$). The interaction between these two factors is not significant. The lower the density and the greater the age of the animals at the beginning of the experiment, the higher the proportion of hermaphrodites that develop.

Ninety-six of the 112 males used in the isolation experiment lived through the 38 day period of observation, 54 from families with more females than males and 42 from families with a very high male sex ratio. The peak period of male to hermaphrodite sex change was between 7 and 15 days after isolation, during which time 76% of the males developed female gonads.

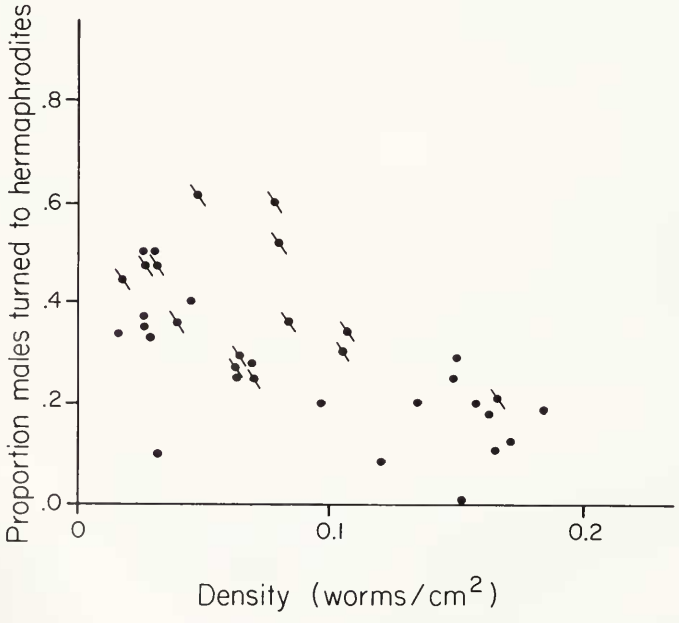

FIGURE 1. Proportion of males turned to hermaphrodites when all-male groups are cultured at varying densities. Each point represents an all-male, non-inbred family group of variable age. ● represents families which were over 70 days of age when the experiment commenced.

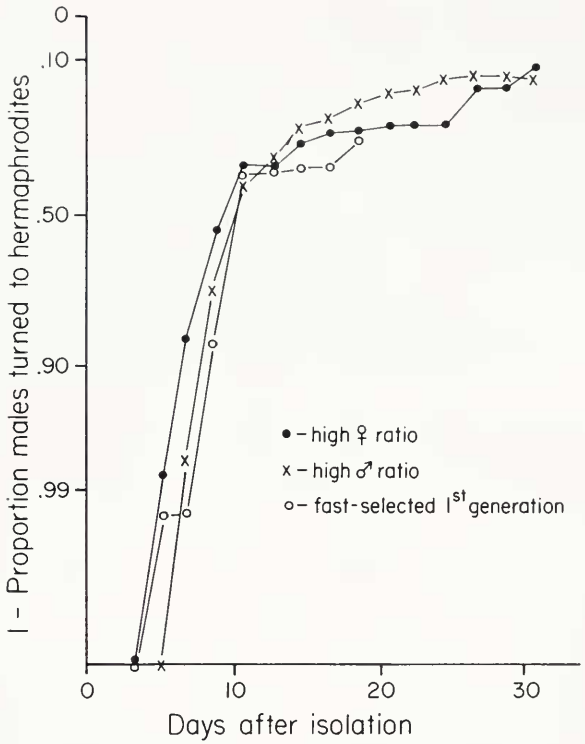

FIGURE 2. Probability plots of proportion of males turned to hermaphrodites in the high male ratio group, high female ratio group and fast-selected first generation against time in days.

Figure 2 shows that there is little observable difference in the proportion of males developing into hermaphrodites between the 2 types of families (high and low male) used as parents. Tests of proportions (Sokal and Rohlf, 1969) between the different sex ratio groups were not significantly different ($P > .05$) for 14 out of 16 2-day intervals during which change was occurring. Twenty-seven out of 32 animals survived the 20-day period of observation in the first selected generation. Tests on the proportion of males switching to hermaphrodites over the 2-day intervals through 20 days show that 4 of the 9 intervals tested are significantly different between the selected generation and the pooled parent generation. Three of the 4 intervals were during the peak period of change from 7–15 days. Surprisingly, the fast-selected line switched later than the pooled parental lines.

It was observed that more food, in the form of the particulate matter (not compacted into fecal pellets) was present at the end of the weekly food replacement cycle in the low density and isolated cultures.

DISCUSSION

Low density promotes the development of female gonads in male *Capitella* sp. I resulting in simultaneously hermaphroditic animals. How this aspect of the environment (low population density) produces such an effect may be explained in two ways. Low density and isolation may be promoting the sex change through increased

food availability or the phenomenon may be induced through the absence of inhibitory effects from animals in close proximity which have female gonads.

The individually isolated animals had a constant and abundant food supply and in the density experiments, the low density cultures had a high amount of food available per animal. In both instances there would be an excess of nutrients available and extra energy might be channeled into the development of the second, female set of reproductive structures and gametes without detracting from any part of the normal energy budget of an animal. This is assuming that the budget is not time-limited. Also, in both experiments only males were used and the results could have been affected by the absence of animals with female gonads. It remains to be seen whether a lack of hormonal secretions in the environment, such as pheromones produced by the females, will trigger the development of female gonads in males. The mechanism by which the age of an animal may affect its ability to become hermaphroditic is also unknown.

The results of the isolation experiment appear to show that possible differences in sex determining genotype do not play a role in the response of males to isolation. If differences in sex-determining genes actually exist in the animals used, they do not affect the rapidity of the male sex change in isolation. However, the ability to actually change sex is genetically controlled and can be selected for, as shown by the fact that inbreeding causes selection for the ability to undergo sex change. The fact that some animals never changed sex in the isolation experiment implies that there is an underlying genetic basis to the expression of the phenomenon.

Since *Capitella* sp. I is a widely dispersed opportunistic species, animals occasionally find themselves at very low densities where finding a mate of the opposite sex would be unlikely. The genes controlling the ability to change sex may, therefore, be selected for in *Capitella* as an 'emergency adaption.' The development of two sets of reproductive structures, although potentially energetically expensive, would increase the chances of finding a suitable mate. The ability to self-fertilize would, of course, be advantageous as well, but there is little evidence that *Capitella* sp. I self-fertilize and the pronounced inbreeding effects in this species are further evidence that selfing is uncommon. In conclusion, it seems that the establishment or maintenance of hermaphroditism as a reproductive strategy may be selected for because of the opportunistic tendencies of this animal.

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